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STUDIES ON THE PARASITIC WASP, *HADROBRACON* *BREVICORNIS* (WESMAEL).

I. GENETICS OF AN ORANGE-EYED MUTATION AND THE PRO- DUCTION OF MOSAIC MALES FROM FERTILIZED EGGS.

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INTRODUCTION.

In a previous paper (1918) I gave a brief account of the general biological relationships and the method of sex-determination in the parasitic wasp, *Hadrobracon brevicornis* (Wesmael). The experiments there reported were begun in the fall of 1916 and continued through the winter. Difficulties in rearing the host caterpillars in sufficient quantity induced me to abandon the work on the wasp and to concentrate on the host, the Mediterranean flour-moth, which was also being investigated genetically. When these technical difficulties were overcome, the work with *Hadrobracon* was renewed and has been continued since June, 1919. During the summer of 1919, the wasps were bred at the Marine Biological Laboratory, Woods Hole, Massachusetts; during the winter of 1919-20, at Franklin and Marshall College, Lancaster, Pennsylvania; and during the summer of 1920 at the Carnegie Station for Experimental Research, Cold Spring Harbor, Long Island. The work is being continued at St. Stephen's College, Annandale-on-Hudson, New York.

My grateful acknowledgments are due for the hospitality of the Woods Hole Laboratory and of the Carnegie Station at Cold Spring Harbor. I am also indebted for financial aid to the Carnegie Institution of Washington and to the American Association for the Advancement of Science from which I have received two grants for research. Mrs. Whiting has given me valuable assistance in the course of the work.

A type of reproduction in which virgin females produce only males while mated females may produce either males or females

has been shown to occur in several groups. Such "male-producing" species are called arrenotokous and it has been supposed that all males arise from unfertilized eggs, females from fertilized. This theory bears the name of Dzierzon (1845) who first applied it to the honey-bee. The males produced by mated females are assumed to develop from eggs into which spermatozoa have not penetrated. Cytological observations showing that males of several arrenotokous species are haploid, while females are diploid tend to corroborate the theory derived from breeding tests.

The only published experimental work on arrenotokous forms showing the method of inheritance of any character difference, other than that of sex, appears to be in crosses of different races of honey-bees. "Criss-cross" inheritance is the rule; the males resemble the mother, while the females inherit characters from both parents. Cross-bred females produce drones showing segregation in expected one to one ratio. From the similarity of these phenomena to sex-linkage, this method of inheritance may be called *sex-linkoid*.

PRESENTATION OF DATA.

(a) *Black-eyed Stocks*.—The results in reference to sex-determination published in my previous paper (1918) showed that the stock of *Hadrobracon brevicornis* obtained in Philadelphia at that time was arrenotokous. The data may be briefly summarized as follows:

Sixty-one females set with males produced 683 males and 918 females.

Nine females set with males produced 197 males, no females.

Thirty-six virgin females produced 1,133 males, no females.

Impaternate sons were crossed back to their virgin mothers and in this way closely inbred daughters were obtained.

Another stock of *Hadrobracon* was secured in Philadelphia in December, 1917, and three virgin females were isolated, producing in all 244 males. One of these females was later mated to one of her sons and produced 9 males and 3 females.

Three males and one female were caught in Mifflintown, Pennsylvania, May, 1920. From eggs laid by this female there

were produced 4 males and 12 females. Seven virgin daughters produced 804 males, and one of these females when later crossed to a son produced 34 males and 5 females.

The Lancaster stock, upon which most of the work with *Hadrobracon* has been done, was derived from a single parasitized caterpillar found in my cultures of the Mediterranean flour-moth at Lancaster in June, 1919. It is highly probable that the caterpillar was stung by a single female which came in from some bakery or grocery store near-by, as I had no cultures of *Hadrobracon* at that time. Seven females were reared, showing that the mother had evidently mated. These females, isolated without males, produced only males which were crossed back to their virgin mothers in order to obtain females. This stock has been bred since that time and has shown considerable variation.

It will be necessary to give only summaries of the typical black-eyed Lancaster stock as the data is very extensive and results in reference to other somatic variations and sex-ratio will be published later.

Fifty-three females, isolated as virgin, produced 5,948 males and no females.

Forty females, set with males, produced 3,944 males, no females.

One hundred thirty-five females, set with males, produced 7,270 males and 4,791 females.

We may now give the entire summaries of the black-eyed stocks, including two different stocks from Philadelphia, one from Mifflintown, and one from Lancaster.

From one hundred and thirty-five virgin females there were 9,242 males.

From forty-nine females set with males there were 4,141 males.

From one hundred and ninety-nine mated females there were 8,000 males and 5,729 females.

I feel very certain that among all these wasps there were none of the orange-eyed variety described below. The difference between the typical black-eyed form and orange is so striking and the eyes of the wasp are so large that such a variation could not escape attention. Moreover the material bred was examined with some care as one of the main purposes of the work was to find genetic differences.

(b) *The Orange Mutation*.—On March 11, 1920, a female, in all respects typical, was isolated as a virgin. Her progeny consisted of 253 black-eyed males and 1 male with orange eyes. This mutant occurred in the first bottle in which the mother had been kept for a period of seven days. The count from this bottle on March twenty-seventh numbered 46 including the mutant. The cultures were at that time developing in the incubator at thirty degrees centigrade at which temperature the period from oviposition to eclosion is about ten days. The mutant may have emerged a few days before the count was made. In any case the egg from which he came was laid within the first seven days of the adult life of the mother which lived until April 1. The mutation may have occurred during the development of a single egg; at least it could not have taken place many cell generations before maturation.

The compound eyes of the mutant were rather light orange. They became slightly darker with age. He was kept alive until May tenth and was mated to various females. "Orange" eyes as they have appeared in later generations are not necessarily orange. They are found to vary from very light cream to deep red and from yellowish orange to pink. No trace of a black fleck such as occurs in the eye of *Drosophila* has been found. The peripheral ommatidia appear black, but this is due, as sections show, to black pigment in the underlying and adjacent integument. Black pigment is also entirely lacking from the ocelli, although here again it occurs in the adjacent integument. In the typical wasp the eyes, both compound and simple, are always jet black. Variations in orange are not considered in the present paper which is concerned only with the difference between black and orange.

(c) *Sex-linkoid Inheritance of Orange*.—The mutant was crossed successfully with six black females and offspring were reared as follows:

Culture No.	Black Males.	Black Females.
178.....	81	94
179.....	49	65
180.....	73	87
182.....	39	68
183.....	162	67
185.....	1	2
Total... 6.....	405	383

By expectation from sex-linkoid inheritance the daughters should carry orange, the sons should be without it. The dominance of black was complete. Four isolated daughters bred as follows:

Culture No.	Origin.	Black Males.	Orange Males.
194.....	178	94	129
198.....	178	90	91
199.....	180	16	25
202.....	179	68	81
Total...4.....		268	326

The excess of orange over the expected one to one ratio indicates that orange is not deficient in viability.

Eight daughters that had mated with their brothers bred as follows:

Culture No.	Origin.	Black Males.	Orange Males.	Black Females.
188.....	178	48	32	77
189.....	"	30	43	51
190.....	"	26	32	70
191.....	"	23	14	49
192.....	"	65	68	58
196.....	"	26	26	50
197.....	"	37	21	63
212.....	183	2	3	7
Total 8.....		257	239	425

The failure of orange to appear in the females is to be expected from sex-linkoid inheritance.

The mutant orange male was crossed successfully to three of his heterozygous daughters and produced offspring as follows:

Culture No.	Origin.	Black Males.	Orange Males.	Black Females.	Orange Females.
193.....	178	52	66	27	36
200.....	180	81	75	13	16
201.....	179	88	102	4	7
Total ..3.....		221	243	44	59

Here in accordance with expectation the orange-eyed females appeared for the first time. In eye color they resembled the

orange males and in later generations have shown apparently the same range of variability. The numerical deficiency of females as compared with their brothers is in this case probably due to the age of the mutant when mating occurred and a consequent scarcity of spermatozoa.

The mutant was set with four of his orange granddaughters, but only a single female was obtained. The record of offspring is as follows:

Culture No.	Origin.	Orange Males.	Orange Females.
209.....	193	211	0
211.....	193	120	0
312.....	201	136	1
313.....	201	88	0

The single orange female from mating 312 was crossed to one of her brothers and produced 35 orange males and 47 orange females. Twenty-five of these orange females, isolated as virgin, produced 2,549 orange males. By back-crossing some of these males to their virgin mothers, orange females were produced and a stock obtained which has since bred true.

Further crosses involving orange may now be summarized.

Twenty-nine black females from various homozygous black stocks crossed with various orange males, produced all black, 1,369 males and 665 females.

Two pairs of heterozygous females by black males produced 28 black males, 26 orange males, and 39 black females.

Twenty-six heterozygous females derived from various crosses of black by orange produced only males, 967 black and 996 orange.

Seven other heterozygous females crossed with orange males produced 170 black males, 151 orange males, 82 black females and 99 orange females.

Thirty virgin orange females derived from various sources produced 2,996 orange males, no females.

Twenty-eight orange females set with males produced 3,365 orange males, no females.

Forty-seven orange females, from various sources mated with orange males likewise from various sources, but not from the cross of orange female by black male, produced only orange, 2,333 males and 1,002 females.

The progeny from orange males derived from orange females crossed with black males are summarized below.

The inheritance is thus far seen to be quite in accordance with expectation.

The case must be mentioned of the occurrence of a single orange male in a cross (237) involving only type stock. There were produced 115 black males and 83 black females besides this orange male. This was possibly a second mutant as it is highly improbable that contamination of the culture could have taken place.

(d) *The Occurrence of Anomalous Males.*—The cross of black male by orange female remains to be considered. Black daughters and orange sons are to be expected. Thirty-three such crosses have been made and the results are summarized in Table I. Eleven agree with expectation, giving 183 black females and 445 orange males. The other twenty-two, however, produced in addition to 816 black females and 889 orange males, 57 *black males*.

It has been shown above that the sons of virgin orange females have always been orange. These anomalous blacks must then have derived their eye color from the sperm nucleus, and consequently must have developed from fertilized eggs.

Anomalous black males and some of their orange brothers were tested by mating to orange females. Table II. shows the results of these tests. Seven black males produced orange daughters only, showing that although their eyes were paternal in origin their gonads were from maternal nuclei. They are therefore mosaics. One of these males, No. 2, was mated successfully to five orange females and sired in all 118 orange daughters.

Five black males produced black daughters only and hence their gonads as well as their eyes were of paternal tissue. One of these, No. 8, was tested with two females.

Eighteen orange males sired orange daughters only. These males were therefore produced in regular manner from unfertilized eggs or, if sperm nuclei entered, they took no part in the formation of eyes or gonads.

One orange male, No. 17, produced 19 black daughters, thus

showing that although his eyes were orange his gonads were paternal in origin.

From the foregoing data it is to be noted that, while black females may be heterozygous and thus diploid for the orange locus, males produce one type of sperm only, which may carry either black or orange but not both.

TABLE I.
PROGENIES FROM BLACK MALES BY ORANGE FEMALES.

Culture No.	Black Males.	Orange Males.	Black Females.
205.....	4	65	41
207.....	6	67	61
214.....	1	28	34
219.....	3	85	67
281.....		11	14
282.....	1	35	42
289.....	3	23	21
340.....	1	8	58
342.....	2	39	45
343.....	6	49	47
344.....	2	86	77
381.....		161	38
384.....	1	62	64
387.....	1	13	11
388.....	1	30	34
391.....	4	42	13
393.....	3	38	42
394.....		36	21
395.....		20	22
396.....	1	17	15
397.....	3	42	34
398.....	2	31	16
399.....		4	1
400.....	1	37	22
401.....		16	8
402.....	2	34	17
403.....		13	16
404.....		26	34
405.....	4	36	32
407.....		2	2
408.....		4	2
409.....	5	22	23
554.....		152	25
Total 33.....	57	1334	999

When pairs of wasps are set together it often happens that the female will refuse to mate and will kick the male away, bending her abdomen down and forward. Her eggs then produce only males as expected. The same male, set with another female or with the same female at another time may succeed in siring

TABLE II.
TESTS OF ANOMALOUS MALES AND ORANGE BROTHERS.

Males.			Offspring.		
No.	Origin.	Character.	Orange Males.	Orange Females.	Black Females.
1	219	Orange	81	63	
2a	219	Black	167	28	
2b	"	"	30	24	
2c	"	"	66	24	
2d	"	"	75	35	
2e	"	"	10	7	
3	282	"	83	40	
4	342	Orange	112	58	
5	"	"	10	8	
6	"	"	65	58	
7	343	Black	24		3
8a	344	"	192		2
8b	"	"	58		19
9	"	Orange	4	2	
10	384	"	10	11	
11	"	"	6	14	
12	"	"	11	17	
13	"	"	19	26	
14	387	Black	44	41	
15	388	"	35		1
16	391	"	100	64	
17	"	Orange	67		19
18	"	"	37	14	
19	393	Black	45	1	
20	"	Orange	54	30	
21	"	"	23	34	
22	"	Black	104	65	
23	"	Orange	36	17	
24	"	"	90	48	
25	396	Black	94		1
26	397	Orange	81	73	
27	"	"	173	1	
28	"	Black	191		1
29	"	Orange	87	52	
30	"	"	3	6	
31	409	Black	21	3	
Totals.....			902	532	—
18 Orange males			67	—	19
1 Orange male			745	332	—
7 Black males			594	—	27
5 Black males					

daughters. Male broods, therefore, do not necessarily mean that the male is sterile. In the case of anomalous males, however, an apparently successful mating frequently results in no daughters or as Table II. shows, in very few, although the female may be highly fertile as indicated by the number of sons produced. Data in reference to this matter are not yet extensive enough for a thorough analysis, and it is therefore thought best to defer publication until later.

DISCUSSION.

(a) *Sex-linkoid Inheritance in the Honey-bee*.—Johannes Dzierzon (1845) based his theory of the parthenogenetic origin of drones of the honey-bee, in part at least, upon crosses of various races. Clear evidence of sex-linkoid inheritance in the bee has been recently furnished by Newell (1914) who crossed a yellow Italian queen to a gray Carniolan drone. All the offspring were yellow. The reciprocal cross produced yellow females and gray males. Heterozygous females produced yellow and gray drones in equal numbers.

Many investigators have crossed Italian bees to the black French or German forms. Sex-linkoid inheritance appears to be the rule here also but there are numerous apparent exceptions.

Perez (1879) crossed yellow Italian queen to black French drone and examined three hundred drone offspring. Forty-nine showed markings interpreted as of paternal origin.

Cuénot (1909) crossed black female to yellow male and obtained females, all of which had yellow bands; three hundred black males; twelve males with small amount of yellow; and two males with broad yellow bands.

Inasmuch as drones intergrading between black and yellow occur it is natural to suppose that there is more than one factorial difference between the two races. It has been suggested that those males which were apparently more or less patroclinous might have arisen from eggs laid by hybrid workers, or that the queen might have been of mixed origin. It may be, however, that the intergrading drones were mosaics, comparable to the mosaic males of *Hadrobracon*.

(b) *Sex Determination and Parthenogenesis*.—In most animals there appears to be a sex-difference in chromatin content. This varies, from a theoretical minute portion of one chromosome to a clearly visible difference of one or more chromosomes. In the case of haploid parthenogenesis, the male has the haploid number, and reduction of the egg nucleus takes place in a normal manner. This occurs apparently in at least most of the Hymenoptera, in the white fly (Stoll, 1919; Schrader, 1920) and probably in several other forms. Diploid parthenogenesis occurs in many

animals. It may result in the production of either males or females, but in Hymenoptera it results in females only, the males being haploid, with the possible exception of the saw flies (Doncaster, 1907). Goldschmidt (1917) has given a convenient classification of types of parthenogenesis.

From the work already done on *Hadrobracon* it appears that parthenogenesis is strictly haploid and therefore arrenotokous. The females are certainly diploid and arise in all cases from fertilized eggs. But it does not therefore follow that all males come from unfertilized eggs, or that such males as arise from fertilized eggs are diploid.

The suggestion has been made that males in Hymenoptera might occur, having the number of chromosomes diploid, except for the sex-chromosome, which might occasionally pass out in the abortive division of the first spermatocyte. Fertilization by the resulting spermatozoön would result in diploid males. In *Hadrobracon* all males, thus far studied, even the anomalous males, appear to be haploid for the orange locus.

(c) *The Bearing of Mosaics on Problems of Fertilization.*—An interesting problem in the occurrence of anomalous, mosaic males centers in fertilization. In the physiological sense, fertilization means the stimulation necessary to initiate development. In forms which are normally parthenogenetic, this stimulation is unnecessary and the entrance of the spermatozoön into the egg can hardly be called physiological fertilization. The egg is already fertile before the appearance of the male cell. In the genetic sense, however, fertilization denotes the union of paternal with maternal germ plasm, or amphimixis. In this sense an egg producing an anomalous male is fertilized, for paternal germ plasm is brought into it, although this is not followed by fusion of nuclei, as is usually the case.

In order to explain sex-mosaics or gynandromorphs it has been suggested that a paternal nucleus might fuse with a maternal, the diploid product giving rise to the female parts of the embryo. Male parts are assumed to be derived from haploid nuclei of paternal, maternal or mixed origin. Polyspermy has been suggested by Morgan (1905) to explain the Eugster gynan-

dromorph bees, but Boveri (1915) contended that the male parts were of maternal color and held that a single sperm nucleus had fused with one of the products of parthenogenetic cleavage. Morgan's hypothesis apparently fits the von Engelhardt bees (1914) in which the male parts resembled the father. Cleavage of a single sperm nucleus, however, might be followed by subsequent fusion of one of the products with the female pronucleus and a result would be obtained similar to Morgan's hypothesis of polyspermy. It is also obvious that haploid nuclei might be derived from both parents and the resulting male parts would then be of mixed origin.

Doncaster (1914) has demonstrated the existence of binucleated eggs in *Abraxas*; and it has been suggested that if one of these contained the sex-chromosome and the other lacked it, their simultaneous fusion with two sperm nuclei would result in a gynandromorph.

Morgan and Bridges (1914) have shown that in *Drosophila* gynandromorphs and other mosaics result from dislocation of a sex-chromosome during cleavage or embryonic development. The male parts are here of course diploid for autosomal factors.

Mosaics due to somatic mutation have likewise been reported in several plants and animals.

It is to be noted that the mosaic males reported in the present paper result from the cross of orange female by black male. The reciprocal cross has failed to give any anomalous results. Whether the production of anomalous males is necessarily correlated with the fact that orange comes in from the mother, black from the father; or whether black females may be obtained which will give anomalous results when crossed to orange males, is uncertain. In the former case the orange factorial difference would itself be responsible for the production of anomalous males. In the latter case the production of irregularities would perhaps depend upon a factorial difference other than that of orange. The answers to this question and to others raised in the present paper may be given by investigations now in progress.

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